

-continued

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Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
      65              70              75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
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Tyr Ser Thr Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
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Ile Lys Arg

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<220> FEATURE:
<223> OTHER INFORMATION: sequence is synthesized

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<400> SEQUENCE: 8

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Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
      35              40              45

Glu Trp Val Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr
      50              55              60

Ala Ala Asp Phe Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser
      65              70              75

Lys Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
      80              85              90

Thr Ala Val Tyr Tyr Cys Ala Lys Tyr Pro His Tyr Tyr Gly Ser
      95              100             105

Ser His Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr
      110             115             120

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Val Ser Ser

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The invention claimed is:

1. A method of purifying a protein which comprises a C_H2/C_H3 region, comprising subjecting a composition comprising said protein to protein A affinity chromatography at a temperature in the range from about 10 ° C. to about 18 ° C.
2. The method of claim 1 further comprising exposing the composition subjected to protein A affinity chromatography to a protease inhibitor.
3. The method of claim 2 wherein the protease inhibitor is EDTA or 4-(2-aminoethyl)-benzenesulfonyl-fluoride, hydrochloride (AEBSF).
4. The method of claim 2 comprising adjusting the pH of the composition prior to protein A affinity chromatography to a pH in the range from about 2.5 to about 3.5.
5. The method of claim 1 wherein the protein is an antibody.
6. The method of claim 5 wherein the antibody binds an antigen selected from the group consisting of HER2, vascular endothelial growth factor (VEGF), IgE, CD20, CD40, CD11a, tissue factor (TF), prostate stem cell antigen (PSCA), interleukin-8(IL-8), epidermal growth factor receptor (EGFR), HER3, HER4, $\alpha4\beta7$ and $\alpha5\beta3$.
7. The method of claim 5 wherein the antibody is selected from the group consisting of Trastuzumab, humanized 2C4, humanized CD11a antibody, and humanized VEGF antibody.
8. The method of claim 5 wherein the antibody binds HER2antigen.
9. The method of claim 8 wherein the antibody is Trastuzumab or humanized 2C4.
10. The method of claim 1 wherein the protein is an immunoadhesin.
11. The method of claim 10 wherein the immunoadhesin is a TNF receptor immunoadhesin.
12. A method of purifying a protein which comprises a C_H2/C_H3 region comprising:
 - a. subjecting a composition comprising said protein to protein A affinity chromatography to provide a recovered composition and measuring leached protein A in said recovered composition;
 - b. if greater than about 20 ng protein A per mg of said protein is measured in said recovered composition, then performing subsequent purification of compositions comprising said protein by protein A affinity chromatography at a temperature in the range from about 10° C. to about 18° C., such that protein A leaching is reduced; and
 - c. exposing the composition subjected to protein A affinity chromatography to a protease inhibitor in order to reduce the protease activity and further reduce leaching of protein A.

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